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6. AUTHORS
Karsten Hazlett

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14. ABSTRACT
The goal of this project is to identify bacterial protein and/or lipid markers of cultivation history. In the long run, such information could be
used to determine weather a bacterium was previously cultivated in the laboratory or grown in the bacterium's natural environment. In
furtherence of this goal, we are building protein- and lipid-compostion databases (comprised of three biological replicates) of Francisella

15. SUBJECT TERMS
Francisella, proteomics, lipidomics, Acinetobactet

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Report Title

Final Report for "Protein, Lipid, Chemical and Structural Signatures of Differentially-Cultivated Francisella tularensis and Acinetobactor baumannii./Research Area 8.3/ TPOC - Dr. Wallace G. Buchholz "

ABSTRACT

The goal of this project is to identify bacterial protein and/or lipid markers of cultivation history. In the long run, such information could be used to determine weather a bacterium was previously cultivated in the laboratory or grown in the bacterium's natural environment. In furtherence of this goal, we are building protein- and lipid-compostion databases (comprised of three biological replicates) of Francisella tularensis (Ft) and Acinetobactor baumannii (Ab) grown under four representative standard lab conditions (SLCs) and three of the bacterium's native environmental conditions (NEC). We are also sequencing the genome of our Ab to facilitate proteomic analysis of this strain.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
03/05/2014	6.00	M. R. Pelletier, Y. Doi, R. K. Ernst, L. G. Casella, J. W. Jones, M. D. Adams, D. V. Zurawski, K. R. O. Hazlett. Unique Structural Modifications Are Present in the Lipopolysaccharide from Colistin-Resistant Strains of Acinetobacter baumannii, Antimicrobial Agents and Chemotherapy, (07 2013): 0. doi: 10.1128/AAC.00865-13
07/08/2013	3.00	Bibiana V Iglesias, Constantine Bitsaktsis, Giang Pham, James R Drake, Karsten R O Hazlett, Kristen Porter, Edmund J Gosselin. Multiple mechanisms mediate enhanced immunity generated by mAb-inactivated F. tularensis immunogen, Immunology and Cell Biology, (12 2012): 0. doi: 10.1038/icb.2012.66
07/08/2013	4.00	Anju Singh, Tabassum Rahman, Meenakshi Malik, Anthony J. Hickey, Cynthia A. Leifer, Karsten R. O. Hazlett, Timothy J. Sellati, Yousef Abu Kwaik. Discordant Results Obtained with Francisella tularensis during In Vitro and In Vivo Immunological Studies Are Attributable to Compromised Bacterial Structural Integrity, PLoS ONE, (03 2013): 0. doi: 10.1371/journal.pone.0058513
07/08/2013	2.00	J. A. O'Hara, L. A. Ambe, L. G. Casella, B. M. Townsend, M. R. Pelletier, R. K. Ernst, R. M. Q. Shanks, Y. Doi. Activities of Vancomycin-Containing Regimens against Colistin-Resistant Acinetobacter baumannii Clinical Strains, Antimicrobial Agents and Chemotherapy, (02 2013): 0. doi: 10.1128/AAC.02501-12
08/22/2012	1.00	Y. Li, D. A. Powell, S. A. Shaffer, D. A. Rasko, M. R. Pelletier, J. D. Leszyk, A. J. Scott, A. Masoudi, D. R. Goodlett, X. Wang, C. R. H. Raetz, R. K. Ernst. LPS remodeling is an evolved survival strategy for bacteria, Proceedings of the National Academy of Sciences, (05 2012): 0. doi: 10.1073/pnas.1202908109
TOTAL:	**5**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received		Paper
07/08/2013	5.00	Mark Pelletier, Leila Casella, Jace Jones, Mark Adams, Daniel Zurawski, Karsten Hazlett, Yohei Doi, Robert Ernst. Unique Structural Modifications are Present in the LPS from Colistin-Resistant Strains of Acinetobacter baumannii, Antimicrobial Agents and Chemotherapy (04 2013)
TOTAL:	**1**	

Number of Manuscripts:

Books

Received	Paper
TOTAL:	

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Karsten Hazlett	0.15	
Bob Ernst	0.05	
Steve Kron	0.04	
Kolbrun Krisjandottir	0.00	
FTE Equivalent:	**0.24**	
Total Number:	**4**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: .....

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):.....

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:.....

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense .....

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: .....

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Don Wolfgeher	0.15
Bryan Shaver	0.50
FTE Equivalent:	**0.65**
Total Number:	**2**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

EXECUTIVE SUMMARY: The goal of this 5.5 yr. project that was sequestered after 2.5 yrs, is to identify bacterial protein and/or lipid markers of cultivation history. In the long run, such information could be used to determine weather a bacterum was previously cultivated in the laboratory (potentially by someone with ill intent) or grown in the bacterium's natural environment. In pursuit of this goal, we have built protein and lipid databases (containing 3 biological replicates) of Francisella tularensis (Ft) and Acinetobactor baumannii (Ab) grown under 4 representative standard lab conditions (SLCs) and 3 of the bacterium's native environmental conditions (NEC). We also sequenced the genome of our Ab strain (TBE 431) to facilitate proteomic analysis of this bacterium.

Progress-to-date: We have completed two biological-replicate proteomes of Ft LVS grown under three SLC (each proteomic analysis entails ~4000 measurements determined in technical duplicate). We are detecting ~1100 proteins (~65% of Ft's potential proteome), which compares very favorably with the detection-rate of another proteomics group analyzing lab-grown Ft (Lenco. Proteomics. 2009). A conservative analysis of our data indicates that roughly 10% of the proteome is significantly responsive to growth conditions; the largest changes are on the order of ~ 8 fold. Notably, many of the most responsive proteins are known virulence factors. Further Ft samples representing the first replicate for 2 additional SLCs and 1 NEC are currently being analyzed. We have completed genome sequencing of the Ab strain; this data will allow our Ab proteomics to commence. On the lipidomics side, we have comprehensive LPS-lipid A analysis for Ft grown under 3 SLCs and 1 NEC. These data reveal the anticipated changes in fatty acid composition as a function of temperature but also indicate temperature and media dependent changes in mannosylation – an unusual LPS modification of unknown function in Ft.
We have also recently developed a novel 2D-TLC (2 dimensional thin layer chromatography) MALDI-TOF approach that allows for detection and characterization of both phospholipids and glycolipids (such as LPS) in a single analysis; this technique has significant potential for miniaturization and field-use. In addition to the above, this funding support is acknowledged in 5 published papers; further publications are anticipated.

Technology Transfer